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Berseem clover seeding rate and harvest management effects on forage yields and nutrient uptake in a swine effluent spray field

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Abstract

A 3-year study was conducted on a Prentiss sandy loam near Pheba, Mississippi to determine optimum berseem clover (*Trifolium alexandrinum* L.) seeding rate (SR) for dry-matter (DM) yield and nutrient uptake in an annual clover–perennial bermudagrass [*Cynodon dactylon* L. (Pers.)] sward, fertilized in April to October with swine effluent. Seed of annual berseem clover (cv. 'Bigbee') was drill-seeded in October at 4, 8, 12, 16, 20 and 24 kg ha^{-1} and harvested either twice in April and May (spring) or once in May. Yield of clover harvested twice was less than that harvested once (5410 vs. 7566 kg ha^{-1}), but N and P uptake were greater in the double-harvest regime. Annual clover responses to SR were described by quadratic trends. Pooled across years and harvest regimes, the optimum SR for DM yield was 16·5 kg ha^{-1} and for P, Cu and Zn uptake, it was 15·7, 14·8 and 16·0 kg ha^{-1}, respectively. Bermudagrass DM yield decreased linearly as SR increased by approximately 6·3 and 66·7 kg DM kg seed^{-1} in double- and single-harvest regimes, respectively. For clover–bermudagrass, the optimum SR for DM yield was 14·0 kg ha^{-1}, and for P, Cu, and Zn uptake, it was 15·1, 14·6 and 15·3 kg ha^{-1}, respectively. A SR of 14·0–14·9 kg ha^{-1} and a first harvest of clover in April appeared to optimize hay yields and uptake of nutrients in clover–bermudagrass. Because bermudagrass N requirement is usually met by swine effluent irrigations, overseeding annual clover would chiefly satisfy producer needs for early forage production.

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Introduction

The waste management practice most often used for confined swine (*Sus scrofa domestica*) operations in the south-eastern USA is based on flushing to anaerobic lagoons and irrigation of nearby cropland with the effluent. Irrigation is usually based on farm-specific waste-management plans that are designed to minimize environmental impacts of nutrient loss, particularly P. The cropping method of choice has become hay of bermudagrass (*Cynodon dactylon* L.); however, annual uptake of P and other nutrients is strongly attenuated by its winter dormancy (Osborne *et al.*, 1999; Brink *et al.*, 2005). Soil buildup of certain trace elements, specifically Cu and Zn, has also been reported when bermudagrass is fertilized with swine lagoon effluent (Novak *et al.*, 2004; Sistani and McLaughlin, 2006). Due to the potential for increased dry-matter (DM) yield and nutrient uptake by year-round forage production, haying of winter cover crops overseeded into bermudagrass has been proposed for remediation or control of soil nutrient concentrations (Rowe and Fairbrother, 2003; McLaughlin *et al.*, 2005; Read *et al.*, 2011). The nutrients taken up by the crop are removed when the hay is harvested.

Annual ryegrass (*Lolium multiflorum* L.) is grown widely in the region because it is economical, adaptable to a broad range of soil and climatic conditions and high in nutritive quality (Brink *et al.*, 2001; Lemus, 2009). Among the winter annual legumes, berseem clover (*Trifolium alexandrinum* L.) is a high-quality pasture and hay crop, having growth in November–December (autumn) and March–June (spring/early summer). It can perform well as a winter cover crop because it is erect growing and begins to flower in early May, when conditions are generally favourable for hay curing. Berseem clover cv. 'Bigbee'

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is a winter-hardy variety that produces longer in the spring than other winter annuals (Knight, 1985). The level of winter hardiness in 'Bigbee' gives it the same range of adaptation as arrowleaf clover (*Trifolium vesiculosum* Savi) and crimson clover (*Trifolium incarnatum* L.) (Graves *et al.*, 1996). Dabney *et al.* (1991) evaluated four legume cover crops in north Mississippi and found 'Bigbee' remained vegetative until early May and produced high biomass yield of approximately 7·16 t ha^{-1}, comparable to the highest yield of 7·62 t ha^{-1} in crimson clover. Studies at a commercial swine farm in north Mississippi reported total nutrient uptake was equal (McLaughlin *et al.*, 2005) or greater (Rowe *et al.*, 2006) in bermudagrass–berseem clover than the more commonly used bermudagrass–ryegrass cropping system. The timing and frequency for hay harvests are also critical, as Rowe and Fairbrother (2003) found changing from a one-harvest date to a two-harvest date regime resulted in berseem clover recovering 25% more P, 40% more Zn and 70% more Cu than annual ryegrass. The heavy metals of environmental concern, Zn and Cu, can accumulate in soils receiving swine effluent due to higher rates of application than removal in the harvested forage (Novak *et al.*, 2004; Sistani and McLaughlin, 2006). Evidence of greater Zn and Cu uptake by legume than grass forage on soil with history of poultry manure (Brink *et al.*, 2001) suggests that if soil accumulations of Zn and Cu are of concern, a legume can extract more of the respective elements than ryegrass.

As with all small-seeded legumes, seedling density is critical to the establishment of healthy and productive stands of berseem clover (Wichman *et al.*, 1991; Ball *et al.*, 2002). 'Bigbee' flowers later than most annual clovers, but is a poor reseeder even when allowed to mature (D. Lang, personal communication). Because reseeding is required each year and berseem clover seed is more expensive than the annual ryegrass, a disadvantage is high seeding costs. The recommended seeding rates (SRs) for forage and hay production in the south-eastern USA are 9–18 kg ha^{-1} pure live seed (PLS) drill-seeded and 22·4 kg PLS ha^{-1} for broadcast-planted (Ball *et al.*, 2002). The recommended SR in Mississippi is 11·2 kg ha^{-1} drilled into a prepared seed bed (Kimbrough and Knight, 1976). Based on the price of 'Bigbee' seed in autumn 2011 of approximately $US 8·82 kg^{-1} (Adams-Briscoe Seed Co., Jackson, GA, USA), costs for sowing a hectare are from $97·02 to $158·76 at SR of 11 to 18 kg ha^{-1}, respectively. When grown for hay in a nutrient-recycling forage system, the amount of biomass harvested in spring is of interest and not autumn–winter grazing (Newton *et al.*, 2003). This change in emphasis to a longer growing season without grazing by livestock lowers the seedling density requirement for stand establishment and productive growth. Additionally, the risk of low SR to crop yields from poor seedling establishment can be lessened on swine farms due to their ability to irrigate with effluent after seeding in autumn to improve plant growth. The objectives of this research were to determine the optimum SR for berseem clover harvested in two regimes for DM yield and uptake of N, P, Cu and Zn and to determine any residual effects of clover SR and harvest management on DM yield and nutrient uptake by perennial bermudagrass.

Materials and methods

This 3-year study (2004–2006) was conducted at a commercial swine farm near Pheba, Mississippi (lat. 33·59 N, long. 88·95 W) on a soil mapped as a Prentiss sandy loam (coarse-loamy, siliceous, semiactive, thermic Glossic Fragiudults, Ultisols) with 2–5% slope (USDA-NRCS, 1976). Rainfall records in the vicinity of the experimental field were obtained from the National Weather Service (Figure 1). In the previous 10 years, the producer fertilized common bermudagrass sod using centre-pivot irrigation that pumped swine effluent from single-stage anaerobic lagoons. The field was irrigated from April to October of each year with approximately 6·0 cm each season (McLaughlin *et al.*, 2005). The effluent had nominal nutrient concentrations of 300–420 mg N L^{-1} and approximately 60 mg P L^{-1}, 0·06 mg Cu L^{-1} and 0·22 mg Zn L^{-1}. In the effluent, the N was approximately 84% NH_4/NH_3 and the P was 80% water-soluble ortho-P (Read *et al.*, 2008). The mean annual fertilization rates were approximately 435 kg N ha^{-1}, 76 kg P ha^{-1}, 35 g Cu ha^{-1} and 128 g Zn ha^{-1} (Sistani and McLaughlin,

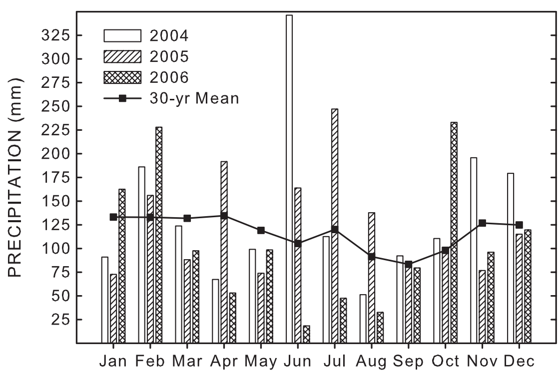

Figure 1 Total monthly rainfall and 30-year average monthly rainfall (1981–2010) recorded by the National Climatic Data Center in the vicinity of the experimental plots (Tibbee, MS, CoopID 228792, approximately 36 km east-southeast of the experimental location).

2006). Timing and amounts of effluent were determined by the farm manager, considering forage needs and requisite drawdown of lagoon effluent. The experimental site was located under one section of the centre-pivot system. In 2003, the existing bermudagrass sod was cleared of weeds or senesced material by mowing to 4-cm stubble height on 9 July, 30 August and 14 September and removing the forage.

Experimental plots were 2 by 4 m, and adjacent blocks were separated by a 2-m alley. The berseem clover treatments, consisting of six SR and two harvest regimes (described below), were arranged in a randomized complete block design with four replicates, giving 48 experimental plots. Each year, a new randomization of treatments was applied to the plot area. By this procedure, plot performance did not reflect any cumulative effects of treatments over more than 1 year. The plot area was prepared for planting by mowing the bermudagrass to a height of 2·5 cm and removing the herbage. Seed of 'Bigbee' berseem clover was inoculated with 'R culture' rhizobia (*Rhizobium leguminosarum* biovar *trifolii)* just before planting. Seed was drilled in nine rows spaced 20 cm apart in late October 2003–2005 at 4, 8, 12, 16, 20 and 24 kg ha^{-1} PLS using an ALMACO (Nevada, Iowa) heavy-duty grain drill equipped with depth bands (~12 mm) on each seeding unit. Based on a test weight of 456 435 seeds kg^{-1}, comparable to the average reported by Graves *et al.* (1996), the SR resulted in 40·6, 81·1, 121·7, 162·3, 202·9 and 243·4 PLS per m of row, respectively. Percentage seedling emergence was determined by counting the number of plants per m of row at three locations in each plot and dividing the average by the number of seeds drilled per m of row. These measurements were obtained approximately 60, 30 and 90 d after planting in 2003, 2004 and 2005, respectively. The two harvest management regimes were two cuttings of annual clover on about 20 April and 15 May and one cutting on about 15 May, hereafter referred to as the double-harvest regime and single-harvest regime, respectively. All harvesting was done using a sickle-bar mower. First harvests in April were made at a cutting height of 9 cm to enable regrowth of the annual clover, whereas all subsequent harvests of clover in May and bermudagrass in summer were at a cutting height of 5 cm. For the single-harvest regime, most clover plants were at early-flowering stage of development, a period in hay production that represents a compromise between increasing herbage yield, decreasing protein content and decreasing digestibility (Joost and Chaney, 1988; Graves *et al.*, 1996). The experimental plots of bermudagrass were harvested three times in 2004 (on 6 July, 5 August and 21 September), twice in 2005 (on 21 July and 13 September) and twice in 2006 (on 17 July and 3 October).

At each harvest, fresh weight yields were recorded for 1- by 4-m swaths cut through the centre of each plot. Forage from the April and May harvests in 2005 was a mixture of berseem clover chiefly and annual ryegrass, and no attempt was made to separate these components. This occurrence of annual ryegrass was presumably due to above average rainfall in spring 2005 (Figure 1). Forage samples of 0·6 kg each were collected in muslin bags, dried at 65°C for 72 h, weighed to determine DM yields for each plot and ground in a Wiley mill to pass a 1-mm screen. Subsamples (~50 g) of the ground forage were stored at room temperature in sealed, plastic vials for subsequent nutrient analysis. Total N concentration was determined from duplicate samples using an automated dry combustion analyzer (Model NA 1500 NC, Carlo Erba[1], Milan, Italy). Herbage P, K, Cu and Zn concentrations were determined by ashing duplicate, 0·8 g subsamples in a ceramic crucible at 500°C for 4 h. The ash was dissolved first in 1·0 mL of 6 M HCl for 1 h, followed by 50 mL of a double-acid solution of 0·0125 M H_2SO_4 and 0·05 M HCl, and the mixture was allowed to stand for 1 h before being filtered through Whatman no. 1 paper. The four nutrient elements were measured by emission spectroscopy on an inductively coupled, dual axial Argon plasma spectrophotometer (ICP-OES, Thermo Jarrel Ash ICP, Iris Advantage ICP, Houghton, MI, USA) (Donohue and Aho, 1992). Certified plant material was used in acid digestion and ICP analysis to verify the quality of the data. Nutrient uptake (kg ha^{-1}) was calculated as the product of DM yield and mineral concentration for each plot at each harvest date in 2004–2006.

Treatment effects on DM yield and nutrient uptake were tested using data for berseem clover harvested once, summed data for berseem harvested twice and summed data for bermudagrass harvested in summer. Total DM yield and nutrient uptake were also calculated by aggregating across sequential harvests (clover + bermudagrass) within each year. Data were analysed within and across years using MIXED program from Statistical Analysis Systems (Littell *et al.*, 1996; SAS Institute Inc, 1999) on a data set that was balanced and complete. In these models, block and year were random effects, and clover harvest regime and SR were fixed effects. A probability level of $P \leq 0{\cdot}05$ was significant and treatment means were compared using Fisher's protected least significant difference (LSD) test. To estimate the optimum clover SR for DM yield and nutrient uptake, regression parameters were estimated using mixed model analysis with covariates (i.e. fitting a polynomial regression over seeding rate) for the harvest data from the 3-year trial ($n = 144$). The random variables in these models were year and block within year. The F test was used to detect difference in linear and quadratic regression parameters (trends) between the double-harvest and single-harvest regimes. When the trend had a significant quadratic regression parameter, the optimum clover SR (independent variable) was determined by solving for the 1st derivative, calculated as $-b_1/2(b_2)$, where b_1 and b_2 are the linear and quadratic regression coefficients, respectively. The 1st derivative of an exponential function gives the point on a relationship where the rate of change in the response variable is zero. Model results are presented for data averaged across 3 years (2004–2006) and, where appropriate, results of statistical tests for a difference in trends between years are discussed based on contrasts (linear and quadratic) within the double-harvest and single-harvest regimes of berseem clover.

Results

Clover seedling emergence

Percentage seedling emergence was approximately 19, 30 and 42% in 2003, 2004 and 2005, respectively (Table 1). Low seedling emergence in autumn 2003 was not associated with low DM yield or P uptake of annual clover in spring 2004, except at the lowest SR of 4 kg ha^{-1} for the single-harvest regime (Figure 2). In autumn 2004, the apparent reduction in percentage seedling emergence as SR increased was probably related to a short, 30-d-time interval between the planting and sampling dates (Table 1).

Forage yields

Annual clover DM yields were 2156 kg ha^{-1} greater in the single- than the double-harvest regime (Table 2). Droughty conditions apparently limited recovery growth after the first cutting in April, as rainfall near the site was below average in March of each year and in April 2004 and 2006 (Figure 1). With low rainfall recorded also in March–May of these years, clover growth at the second harvest was either virtually absent and unharvestable (2004) or greatly reduced (2006). When data were pooled across years and harvest regimes, a quadratic trend described the response of annual clover DM yield to SR, and the trends were similar statistically in both harvest regimes (Table 3, Figure 2). Based on the 1st derivative of this polynomial function, the optimum SR for berseem DM yield was approximately 16·5 kg ha^{-1}. The F test for difference between years in the linear coefficient and quadratic coefficient (each with 2 degrees of freedom) had $P = 0{\cdot}19$ for the double-harvest regime and $P = 0{\cdot}14$ for the single-harvest regime (not presented), indicating regression parameters for each harvest regime were consistent across years.

Bermudagrass DM yields were 1146 kg ha^{-1} greater in the double- than single-harvest regime of annual clover ($P < 0{\cdot}01$) (Table 2). The harvest regime effect was significant each year and had a 5% LSD value ($n = 24$) of 786 kg ha^{-1} in 2004, 1138 kg ha^{-1} in 2005 and 442 kg ha^{-1} in 2006. The clover harvest regime × SR regression interaction effect was significant in both the reduced ($P < 0{\cdot}05$) and full regression models ($P < 0{\cdot}10$) (Table 4). When data were pooled across years, DM yields decreased linearly as clover SR increased by approximately 6·3 kg DM kg seed^{-1} for the double-harvest regime and 66·7 kg DM kg seed^{-1} for the single-harvest regime (Figure 3). Due to these linear trends, the optimum clover SR for bermudagrass DM yield was the minimum SR, and yields predicted at the lowest SR of 4 kg ha^{-1} were approximately 12 357 and 11 815 kg ha^{-1} for the double- and single-harvest regimes, respectively.

Total DM yields (clover + bermudagrass) were 1010 kg ha^{-1} greater in the single- than double-harvest regime (Table 2). Apparently, increased DM

Table 1 Seedling density and percentage seedling emergence of 'Bigbee' berseem clover overseeded into common bermudagrass at six rates in autumn 2003, 2004 and 2005. Values represent the average of four replicate plots in each of two harvest management regimes imposed in spring 2004, 2005 and 2006.

Seeding rate (kg ha^{-1})	Seedling density (plants per m of row)			Seedling emergence (%)		
	2003	2004	2005	2003	2004	2005
4	8·5 e*	16·6 d	16·7 f	21·1 a	41·0 a	41·2 a
8	16·1 d	28·2 cd	34·0 e	19·9 a	34·7 ab	42·0 a
12	24·1 c	39·8 bc	54·2 d	19·8 a	32·7 abc	44·5 a
16	33·9 ab	41·4 bc	64·9 c	20·9 a	25·5 bc	40·0 a
20	32·1 b	48·0 ab	88·7 b	15·8 a	23·6 c	43·7 a
24	40·1 a	61·8 a	103·4 a	16·5 a	25·4 bc	42·5 a

*Means ($n = 8$) within a column followed by the same letter do not differ significantly at the 5% level of probability.

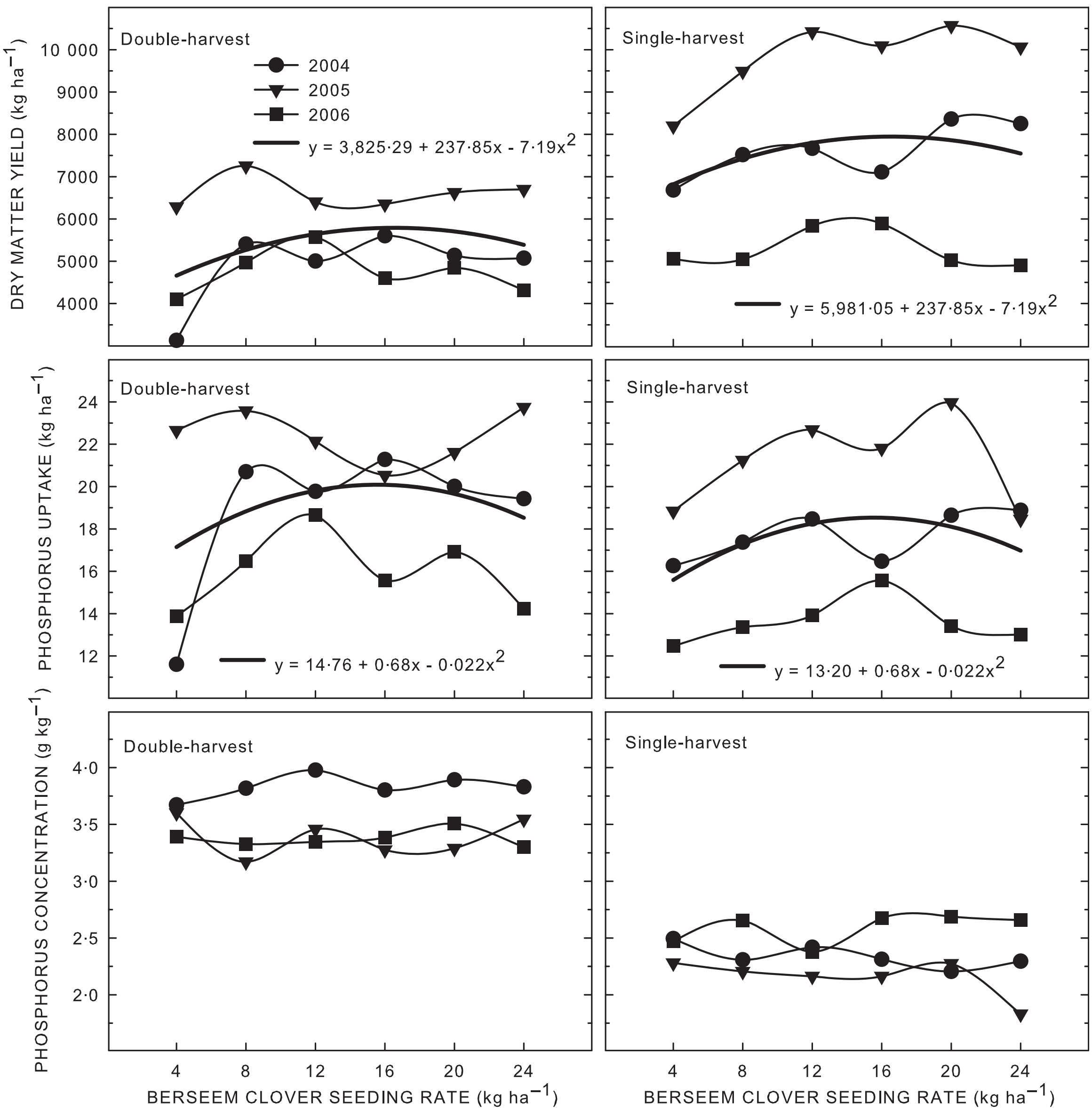

Figure 2 Relationships between forage dry-matter yield, P uptake and herbage P concentration in 'Bigbee' berseem clover and seeding rate of berseem clover harvested twice in spring (April + May) and harvested once in May of 2004, 2005 and 2006. Values within each year represent the mean of four observations. The polynomial regression parameters are based on analysis of data pooled across harvest regimes and years (n = 144).

yield of bermudagrass in the double-harvest regime did not offset less berseem clover yield, so total DM yield was least in the double-harvest regime in 2004 and 2005. The response of total DM to clover SR was described by a quadratic trend (data not presented) and the optimum SR for total DM yield was approximately 14·0 kg ha^{-1}, giving a 2·5 kg ha^{-1} lower SR than observed for the annual clover harvests only.

Nutrient uptake

Berseem clover P concentrations were consistently greater in double- than single-harvest regime (Figure 2), reflecting the harvest of younger plant material. So despite low DM yields of clover harvested twice, P uptake was significantly greater than clover harvested once; analysis of variance tests indicated 18% greater P uptake in 2006 (13·6 vs. 16·0 kg ha^{-1}; $P < 0·01$)

Table 2 Forage dry-matter yield (kg ha^{-1}) of 'Bigbee' berseem clover, common bermudagrass and the summation of these two components averaged across six seeding rates of berseem clover overseeded in autumn and cut in spring as a double-harvest regime of berseem in April and May and as a single-harvest regime of berseem in May of 2004–2006. All the plots were fertilized with swine effluent from April to October.

	Berseem clover		Bermudagrass		Total dry matter	
Year	Double	Single	Double	Single	Double	Single
2004	4892 b*	7598 a	14 021 a	12 807 b	18 913 b	20 406 a
2005	6603 b	9805 a	16 568 a	15 236 b	23 172 b	25 041 a
2006†	4735 b	5294 a	6293 a	5402 b	11 028 a	10 696 a
Average	5410 b	7566 a	12 294 a	11 148 b	17 704 b	18 714 a

*Within a year and a component of the multicrop forage production system, means followed by the same letter do not differ significantly at the 5% level of probability. †Drought year.

Table 3 Regression parameter estimates for berseem clover dry-matter (DM) yield and nutrient uptake response to six berseem clover seeding rates (SRs) averaged across 2004–2006 with harvest-regime-specific adjustments to the Y-intercept, b_0.

Response variable (kg ha^{-1})	Harvest regime (b_0) Double	Single	b_0 effect	SR (b_1)	SR^2 (b_2)	1st derivative †
DM yield	3825·29	5981·05	**	237·85 *	−7·19 **	16·54
N uptake	106·87	85·07	**	6·667 **	−0·205 **	16·26
P uptake	14·76	13·20	*	0·685 NS	−0·0218 **	15·67
K uptake	165·70	172·50	NS	11·323 **	−0·336 **	16·82
Cu uptake	22·44	33·52	**	1·934 NS	−0·0651 *	14·85
Zn uptake	106·41	75·06	NS	3·584 NS	−0·1117 *	16·04

*,**Based on Type 1 sums of squares in the reduced model, the effect of harvest regime (Y-intercept, b_0), linear regression coefficient or quadratic regression coefficient are significant at $P < 0.01$ and $P < 0.05$, respectively; otherwise, not significant (NS). †1st derivative of the polynomial function, where the rate of change in the response variable is equal to zero, was evaluated as $b_1/(2 \times b_2)$.

Table 4 Regression parameter estimates for common bermudagrass dry-matter (DM) yield and nutrient uptake response to six berseem clover seeding rates (SRs) and SR by harvest regime interaction for data averaged across 2004–2006 with harvest-regime-specific adjustments to the Y-intercept, b_0, and the coefficient of linear regression, b_1.

Response variable	Harvest regime (b_0)			SR (b_1)†		Harvest
(kg ha^{-1})	Double	Single	b_0 effect	Double	Single	regime by SR
DM yield	12 382	12 082	*	−6·28	−66·72	*
N uptake	246·38	249·00	*	−0·114	−0·951	NS
P uptake	31·54	30·60	*	−0·0209	−0·0204	NS
K uptake	262·98	256·67	*	−0·378	0·836	NS
Cu uptake	46·54	50·47	t	−0·0278	−0·0434	NS
Zn uptake	178·61	165·74	*	−0·447	−0·069	NS

*,t Based on Type 1 sums of squares in the reduced model, the effect of harvest regime (Y-intercept, b_0), seeding rate (SR, b_1), and harvest regime by SR regression interaction are significant at $P < 0.05$ and $P < 0.10$, respectively; otherwise, not significant (NS). †Unlike berseem clover (see Table 3), the quadratic term for seeding rate, SR^2 (b_2), was not found to contribute significantly to the model fit and was therefore excluded from the final model.

and no significant difference in P uptake between harvest regimes in 2004 and 2005. Similar to P, clover N uptake averaged 26% greater in the double- than single-harvest regime (Table 3), and the largest difference of approximately 36 kg ha^{-1} was observed in 2006.

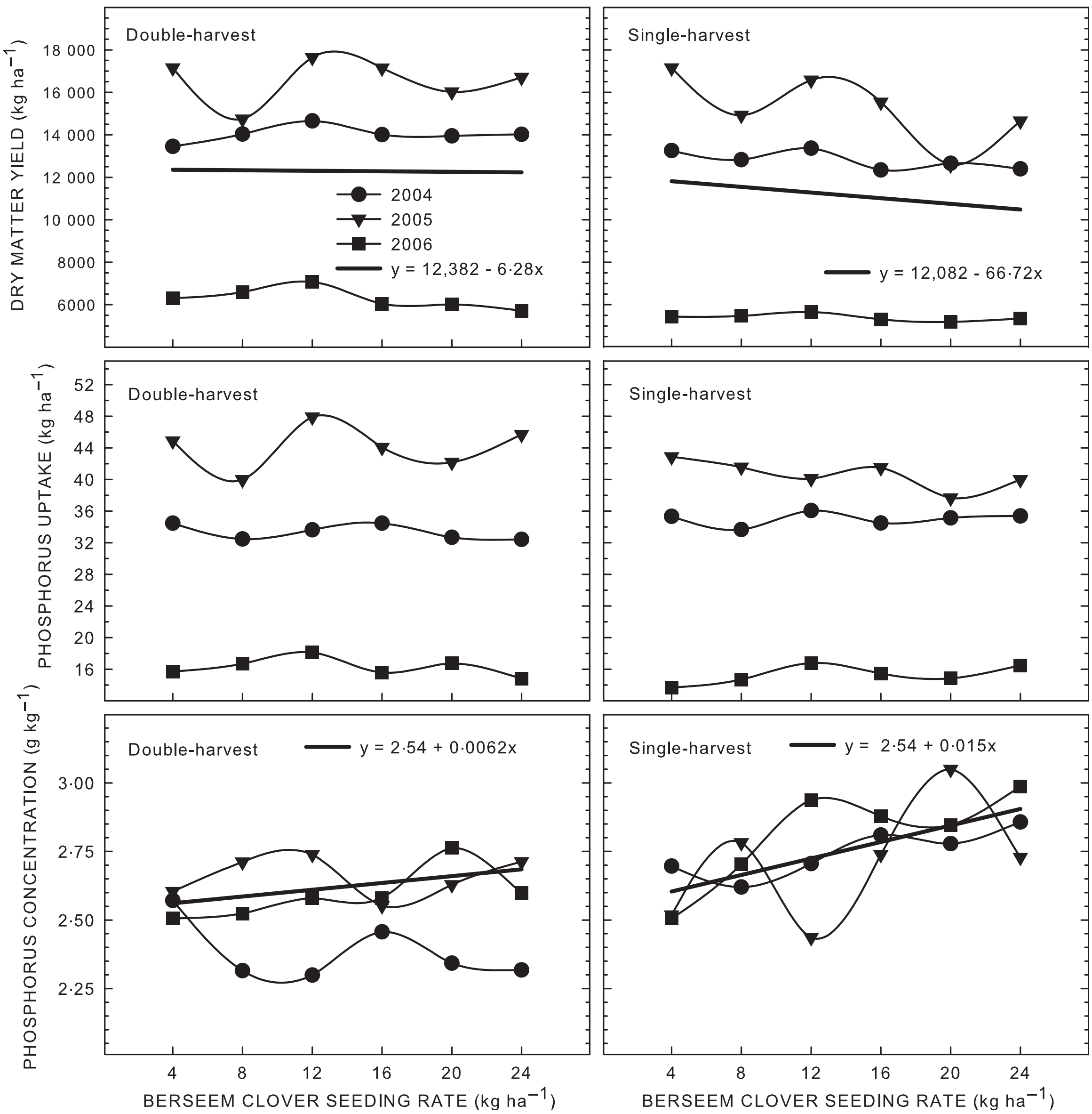

Figure 3 Relationships between forage dry-matter yield, P uptake and herbage P concentration in common bermudagrass and seeding rate of 'Bigbee' berseem clover harvested twice in spring (April + May) or once in May of 2004, 2005 and 2006. Values within each year represent the mean of four observations. The linear regression parameters are based on analysis of data pooled across harvest regimes and years (n = 144).

In berseem clover, nutrient uptake responses to SR were described by quadratic trends that did not differ statistically between harvest regimes (Table 3; Figure 2). The best SR for N, P, K, Cu and Zn uptake was approximately 16·3, 15·7, 16·8, 14·8 and 16·0 kg ha^{-1}, respectively, giving an overall average of 15·9 kg PLS ha^{-1}. The F test for year × SR trend interaction had $P < 0{\cdot}05$ for P, K and Zn uptake in the double-harvest regime and had $P < 0{\cdot}10$ for Cu uptake in the single-harvest regime, suggesting the need for different quadratic regression parameters in each year. Values for P uptake at the optimum clover SR of 15·7 kg ha^{-1} were approximately 20·0 and 18·5 kg ha^{-1} in the double- and single-harvest regimes, respectively (Figure 2).

In bermudagrass and unlike its forage yield response to SR, the responses of N, P, K, Cu and Zn uptake were not described by a linear function and the harvest regime × SR regression interaction effect was not significant (Table 4; Figure 3). Based on Y-intercept (b_0) values, P, K and Zn uptake were significantly greater in the double- than the single-harvest regime, whereas, an opposing trend for treatment difference in Cu uptake had $P = 0{\cdot}06$. The analysis of variance across years and SR indicated P uptake averaged 31 and 30 kg ha^{-1} in the double- and single-harvest regimes, respectively. Among the five nutrients analysed, bermudagrass P concentration was the only one with a significant linear response to clover SR ($P = 0{\cdot}011$; Figure 3) and a significant harvest regime × SR regression interaction effect ($P = 0{\cdot}018$) (data not presented). These results, which indicated forage P concentrations averaged higher and increased faster as clover SR increased ($P < 0{\cdot}05$) in the single- than double-harvest regime (0·015 vs. 0·006 g P kg DM^{-1} per kg seed ha^{-1}), and the larger negative trend in bermudagrass DM yield for single-harvest regime (Table 4), would support the lack of difference in P uptake between harvest regimes. With regard to bermudagrass Zn uptake, analysis of variance across years and SR indicated higher average values for the double- than single-harvest regime (151·9 vs. 143·7 kg ha^{-1}; LSD = 7·7).

Total uptake of N, P and Cu was greater in the double- than the single-harvest regime of berseem clover ($P < 0{\cdot}01$) (data not presented). For total uptake of N, P and K, each of the responses to clover SR was described by a quadratic function; however, the linear coefficient was significant for K uptake only. The best clover SR for total N, P, K, Cu and Zn uptake was approximately 14·7, 15·1, 17·4, 14·6 and 15·3 kg ha^{-1}, respectively, giving an overall average of 15·4 kg ha^{-1}. These observations indicated the optimum SR for total nutrient uptake was lower than for annual clover nutrient uptake by approximately 1·6, 0·6, 0·2 and 0·7 kg ha^{-1} for N, P, Cu and Zn, respectively.

Discussion

Because seeding method and soil type were constant, the amount and distribution of autumn rainfall were probably the most important factors determining seedling density of berseem clover. The use of this small-seeded annual legume as a winter cover crop will require cultural practices that ensure successful stand establishment (Wichman *et al.*, 1991; Ball *et al.*, 2002). In general, a full stand of clover is anything greater than 129 plants m^{-2} (Barnhart, 2008), which in the present study is equivalent to 25·8 plants per m of row. Based on this figure, a 62–65% stand was recorded for a seeding rate of 8 kg ha^{-1} in 2003 and 4 kg ha^{-1} in 2004 and 2005 for data averaged across harvest regimes (Table 1); however, average DM yields for these clover stands were similar to values observed at higher seeding rates ($P > 0{\cdot}40$, see Figure 2). These results are consistent with the seeding rate study of Wichman *et al.* (1991) and illustrate the ability of berseem clover to compensate for inter-row plant density; the widely spaced plants 'filled in' through additional tillering and spreading of root crowns (Ball *et al.*, 2002). Therefore, low seedling density does not necessarily mean low productivity, and an excessive seeding rate that prevents such compensation by plants may not be economical.

Annual clover DM yield differed markedly between years, probably because of high variability in the second harvest of the double-harvest regime. High DM yields observed in 2005 were associated with December–May rainfall of 762 mm, which is comparable to long-term rainfall for this period of 778 mm (Figure 1). Rainfall in winter 2006 also appeared favourable for high DM yields, although clover yields remained significantly less in the double- than single-harvest regime (4735 vs. 5294 kg ha^{-1}). In a Mississippi study, McLaughlin *et al.* (2005) harvested 'Bigbee' berseem clover twice in spring and reported DM yields in the range of 3000 to 4200 kg ha^{-1}, somewhat lower than the range observed in the present study. The Mississippi Natural Resources Conservation Service Practice Standard 359 stipulates that the timing of nutrient application should correspond as closely as possible with plant nutrient uptake (Mississippi NRCS, 2000). Accordingly, effluent is typically applied between April and September when bermudagrass is growing actively. Because irrigation is possible with an actively growing winter cover crop, decreased growth of clover in spring due to low soil moisture may be less of a concern on swine effluent application sites.

Harvesting clover once maximized clover DM yields and total (clover + bermudagrass) DM yields in 2004 and 2005; whereas, harvesting clover twice maximized uptake of P and Zn. For data pooled across years, quadratic trends described the relationship between clover SR and clover DM yield, and the yields predicted at the optimum SR of 16·5 kg ha^{-1} were approximately 5792 and 7948 kg ha^{-1} for the double- and single-harvest regimes, respectively. These results contrast with Rowe and Fairbrother (2003) for 'Bigbee' drilled into dormant bermudagrass at a SR of 16·8 kg ha^{-1}. In that study, DM yields were usually highest for a two-cut system on 1 April and 1 June (average = 9240 kg ha^{-1}) and least for a one-cut system on 1 June (average = 8440 kg ha^{-1}). Differences between studies in harvest regime effects are probably related to a shorter (~30-d) harvest interval for the double-harvest regime in the present study.

While an optimum SR of 16·5 kg ha^{-1} is high within the recommended range of seeding rates, 9–18 kg ha^{-1} (Ball *et al.*, 2002), the use of higher SR in dormant bermudagrass sod was clearly advantageous to growth of 'Bigbee' berseem clover. Seeding rate recommendations are subject obviously to location variation (Ball *et al.*, 2002) and usually include fertilizer applications according to a pre-plant soil test, which was not determined in the present study. In irrigated and rainfed trials in Montana, Wichman *et al.* (1991) evaluated two-cut systems by drilling 'Bigbee' on fallow land in May at rates of 2·25, 4·5, 6·75, 9·00 and 11·25 kg PLS ha^{-1} and row spacings of 15, 30 and 60 cm. They reported a 99% increase in DM yield at the first harvest as seeding rate increased across the three row spacing, no significant effect of seeding rate at the second harvest, and no difference in DM yield for the three highest seeding rates.

Berseem clover harvest frequency and seeding rate also were critical to growth of perennial bermudagrass. Cumulative DM yields in summer were 9–16% greater in the double-harvest than single-harvest regime, depending on study year. Additionally, DM yields increased linearly as clover SR decreased, and the regression coefficient for this relationship was approximately 10-fold less in the double- than single-harvest regime. While these results indicated the minimum clover SR of 4 kg ha^{-1} was best for bermudagrass DM yield, they suggest forage management practices to enable high hay yields in summer can consider increasing the clover SR if the winter cover crop is harvested twice in spring, but not if it is harvested once. Similar to the present study, Rowe *et al.* (2006) reported overseeded berseem clover harvested once on 1 June decreased bermudagrass DM yield by approximately 1720 kg ha^{-1}, as compared to clover harvested twice, on 15 April and 1 June. Growth of the winter cover crop with a closed canopy is expected to limit radiant heating of soil surface and shaded bermudagrass stubble is not expected to break dormancy (Ball *et al.*, 2002). It is speculated that harvesting clover in mid April, combined with the usually poor second harvest in May, opened the canopy during a critical period resulting in breaking of dormancy in bermudagrass. Spring 'greenup' in bermudagrass is an active period of growth and nutrient utilization. For instance, a Mississippi study fertilized common and 'Coastal' bermudagrass with swine effluent and harvested herbage weekly over 60 d in the spring (May–June) and summer (July–August) (Brink *et al.*, 2005). For these season-specific harvest treatments, bermudagrass DM yields and uptake of N, P and K followed a linear trend across the spring harvest dates and a quadratic trend across the summer harvest dates, and maximum uptake of nutrients was greatest in spring. In the present study, evidence of lower optimal SR values for total uptake of N, P, Cu and Zn than for the clover harvests only may reflect the competitive effects of berseem clover on bermudagrass growth and nutrient uptake. In contrast, the best SR for total K uptake exceeded the optimum SR for berseem K uptake by approximately 0·6 kg ha^{-1}, suggesting increased ground coverage of clover improved K concentration, biomass or both of these attributes in the harvested forage.

Increased uptake of N and P by berseem clover harvested twice each spring was associated with higher tissue nutrient concentrations rather than higher DM yields. These results are consistent with evidence that frequent cuttings of berseem clover enable high nutritive value (i.e. higher leaf–stem ratio and digestibility) and less frequent cuttings enable high DM yields (Joost and Chaney, 1988). Averaged across years and seeding rates, herbage P was approximately 3·5 and 2·4 g kg^{-1} in the double- and single-harvest regimes, respectively. Brink *et al.* (2001) reported P concentrations of berseem clover harvested at full bloom of 2·3–2·6 g kg^{-1}, and Wichman *et al.* (1991) reported values of 2·5–3·0 g kg^{-1} in herbage harvested twice. Values for P uptake in the present study generally support Rowe and Fairbrother (2003) although they reported somewhat greater values of 22·2 kg ha^{-1} for a single harvest on 1 June and 29·5 kg ha^{-1} for the combined harvests on 15 April and 1 June. In the present study and at the optimum SR of 15·7 kg ha^{-1}, P uptake was approximately 18·5 kg and 20·0 kg ha^{-1} in the single- and double-harvest regimes, respectively. Moreover, mean values for P uptake (n = 4) were usually less than 24 kg ha^{-1} (Figure 2). Also in contrast to Rowe and Fairbrother (2003), the present study found no significant effect of harvest management on uptake of K and Zn by berseem clover.

Bermudagrass achieved the highest P uptake of approximately 44 kg ha^{-1} in 2005, as compared to 30 kg ha^{-1} when data were averaged across years. Various studies have reported bermudagrass can remove 50–60 kg P ha^{-1} year^{-1} depending on biomass yield and tissue P concentration, which are influenced by a number of factors, including the rate and timing of effluent applications (Brink *et al.*, 2005; Read *et al.*, 2008). In a study on spring N fertilization of three winter cover crops overseeded into bermudagrass that was fertilized in summer with 258 kg N ha^{-1} in swine effluent, Read *et al.* (2011) reported significantly higher P uptake in the berseem clover and winter fallow treatments (average = 22·6 kg ha^{-1}) than plots overseeded with annual ryegrass or cereal rye (*Secale cereale* L.) (average = 19·6 kg ha^{-1}).

Reflecting the negative response of bermudagrass to clover SR, lowering the SR by approximately 0·6 kg ha^{-1} would probably maintain DM yields in summer without greatly reducing total P uptake. Results indicated a similar reduction in clover SR for best total uptake of Cu and Zn by berseem clover–bermudagrass. A reduced seeding rate is generally of most benefit to the grower when the bermudagrass hay produced has high value. Though soil N was not measured, berseem clover is known to produce substantial quantities of N through symbiotic N activity (Williams *et al.*, 1990) and some of this N would likely improve production and/or quality of bermudagrass. In a 2-year study on spring N fertilization of winter annual forages, Read *et al.* (2011) reported bermudagrass DM yield increased as N rate increased in 2000, when just 3·6 cm of effluent (equivalent to 258 kg ha^{-1}) was applied due to below-normal lagoon levels. Additionally, both Read *et al.* (2011) and McLaughlin *et al.* (2005) observed DM yields were numerically greater in bermudagrass–berseem clover, as compared to bermudagrass-winter fallow treatment. Nevertheless, much of the symbiotic N would likely remain in soil, because N in the applied effluent usually meets forage bermudagrass annual N requirement of 200–400 kg N ha^{-1} at moderate-to-high production levels, respectively (Osborne *et al.*, 1999; Read *et al.*, 2008). Therefore, overseeding berseem clover would chiefly satisfy producer needs for early forage production. Knight (1985) reported 'Bigbee' is more productive in autumn and winter than other winter annual clovers except 'Tibbee' crimson clover. In addition, 'Bigbee' continues to produce forage until late May or early June. Spring haying can be problematic, because intermittent cool temperatures and rains will slow curing of the hay, as compared to herbage harvested later in the season. At this location in NE Mississippi, 20 April is the earliest that most winter annuals can be harvested and safely preserved. Haylage is an alternative that enables earlier spring harvests and would, based on results of the present study, favour increased P uptake in annual clover and clover–bermudagrass biomass.

The practice of overseeding berseem clover, a cool-season annual clover, into bermudagrass has several advantages for growers, including the removal of nutrients from soil during winter when bermudagrass is dormant, an earlier hay harvest in spring, and production of high-quality hay (McLaughlin *et al.*, 2005; Rowe *et al.*, 2006). Because annual clover SR influenced the total quantity of hay produced per ha, the use of an optimum SR supports waste management plans on commercial swine farms, where crop removal for a certain yield goal is used in making manure application decisions. The best practice to optimize total dry-matter yield appeared to be a clover SR of 14·0 kg ha^{-1} and a double-harvest regime, with the first cutting in mid April. Additionally, harvesting annual clover twice increased total N and P uptake and slightly increased total Zn uptake. A clover SR of 14·9 kg ha^{-1} appeared to be a best management practice when using berseem clover–bermudagrass for year-round management of the environmentally sensitive nutrients, N, P, Cu and Zn. Because clover seeding rates for optimal clover–bermudagrass hay production were 2·8 to 3·7 kg ha^{-1} higher than the recommended 11·2 kg ha^{-1} rate for conventional clover seedings in Mississippi (Kimbrough and Knight, 1976), our results suggest additional input costs for safe and effective use of swine effluent on berseem clover–bermudagrass stands.

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References

Ball D.M., Hoveland C.S. and Lacefield G.D. (2002) *Southern Forages* 3rd edn. Norcross, GA: Potash and Phosphate Institute and the Foundation for Agronomic Research.

Barnhart S.K. (2008) Evaluating hay and pasture stands for winter injury. Cooperative Extension Service, Iowa State University. Available at: http://www.extension.iastate.edu/Publications/PM1362.pdf (accessed 6 March 2013).

Brink G.E., Pederson G.A., Sistani K.R. and Fairbrother T.E. (2001) Uptake of selected nutrients by temperate grasses and legumes. *Agronomy Journal*, **93**, 887–890.

Brink G.E., Pederson G.A. and Sistani K. R. (2005) Nutrient uptake of swine effluent-fertilized bermudagrass during primary spring and summer growth. *Journal of Plant Nutrition*, **28**, 1337–1346.

Dabney S.M., Buehring N.W. and Reginelli D.B. (1991) Mechanical control of legume cover crops. In: Hargrove W.L. (ed.) *Cover crops for clean water*, pp. 146–147. Ankey, IA: Soil and Water Conservation Society.

Donohue S.J. and Aho D.W. (1992) Determination of P, K, Ca, Mg, Mn, Fe, Al, B, Cu, and Zn in plant tissue by Inductively Coupled Plasma (ICP) emission spectroscopy. In: Plank C.O. (ed.) *Plant analysis reference procedures for the southern region of the United States*, pp. 34–37. Southern Cooperative Series Bulletin 368.

Graves W.L., Williams W.A. and Thomsen C.D. (1996) *Berseem clover: a winter annual forage for California agriculture*. Publication 21536, University of California,

Division of Agriculture and Natural Resources, Davis, CA.
Joost R.E. and Chaney C. R. (1988) Management of berseem clover for optimum forage yield and quality. *Louisiana Agriculture*, **31**, 18–19.
Kimbrough E.L. and Knight W.E. (1976). *Forage: Bigbee berseem clover*. Cooperative Extension Service, Mississippi State University Information Sheet, IS 1306. 2 p. Available at: http://msucares.com/pubs/infosheets/is1306.pdf (accessed 6 March 2013).
Knight W.E. (1985) Registration of 'Bigbee' berseem clover. *Crop Science*, **25**, 571–572.
Lemus R. (2009) Utilization of annual ryegrass. Forage News. pp. 1–5. Cooperative Extension Service, Mississippi State University. Available at: http://msucares.com/crops/forages/newsletters/09/1.pdf (accessed 6 March 2013).
Littell R.C., Milliken G.A., Stroup W.W. and Wolfinger R.D. (1996) *SAS regime for mixed models*, p. 633. Cary, NC: SAS Institute, Inc.
McLaughlin M.R., Sistani K.R., Fairbrother T.E. and Rowe D.E. (2005) Overseeding common bermudagrass with cool-season annuals to increase yield and nitrogen and phosphorus uptake in a hay field fertilized with swine effluent. *Agronomy Journal*, **97**, 487–493.
Mississippi Natural Resources Conservation Service (2000) Waste Treatment Lagoon. Code 359. Field Office Tech. Guide Section IV. Mississippi NRCS, Jackson MS.
Newton G.L., Bernard J.K., Hubbard R.K., Allison J.R., Lowrance R.R., Gascho G.J., Gates R.N. and Vellidis G. (2003) Managing manure nutrients through multi-crop forage production. *Journal of Dairy Science*, **86**, 2243–2252.
Novak J.M., Watts D.W. and Stone K.C. (2004) Copper and zinc accumulation, profile distribution, and crop removal in coastal plain soils receiving long-term, intensive applications of swine manure. *Transactions of the American Society of Agricultural Engineers*, **47**, 1513–1522.
Osborne S.L., William R.R., Gordon V.J. and Rogers J.L. (1999) Bermudagrass response to high nitrogen rates, source, and season of application. *Agronomy Journal*, **91**, 438–444.
Read J.J., Brink G.E., Adeli A. and McGowen S.L. (2008) Swine effluent application timing and rate affect nitrogen use efficiency in common bermudagrass. *Journal of Environmental Quality*, **37**, 180–189.
Read J.J., Brink G.E., McLaughlin M.R. and Sistani K.R. (2011) Nitrogen and winter cover crop effects on spring and summer nutrient uptake. *Grass and Forage Science*, **66**, 381–390.
Rowe D.E. and Fairbrother T.E. (2003) Harvesting winter forages to extract manure soil nutrients. *Agronomy Journal*, **95**, 1209–1212.
Rowe D.E., Fairbrother T.E. and Sistani K.R. (2006) Winter cover crop and management effects on summer and annual nutrient yields. *Agronomy Journal*, **98**, 946–950.
SAS Institute Inc. (1999) *SAS/STAT user's guide, version 8.0, volume 3*. Cary, NC: SAS Institute, Inc.
Sistani K.R. and McLaughlin M.R. (2006) Soil nutrient dynamics from swine effluent application to common bermudagrass overseeded with cool-season annuals. *Journal of Sustainable Agriculture*, **28**, 101–116.
USDA-NRCS (1976) Soil Survey of Clay County, Mississippi. Soil Survey Staff, Natural Resources Conservation Service, United States Department of Agriculture. Web Soil Survey. Available at: http://websoilsurvey.nrcs.usda.gov (accessed 6 March 2013).
Wichman D.M., Welty L.E., Westcott M.P., Ditterline R.L. and Dunn R.L. (1991) *Berseem clover seeding rates and row spacings for Montana*. *Montana AgResearch*. pp. 15–17. Bozeman, MT: Montana State University Agricultural Experiment Station.
Williams W.A., Graves W.L. and Cassman K.G. (1990) Nitrogen fixation by irrigated berseem clover versus soil nitrogen supply. *Journal of Agronomy and Crop Science*, **164**, 202–207.